20.(**amended**) A composition comprising a polymerase or reverse transcriptase including a pair of tags covalently bonded to a site on the polymerase, where a detectable property of at least one of the tags undergoes a change before, during and/or after monomer incorporation.

REMARKS

Applicant intends this response to be a complete response to the Examiner's Non-Final Office Action. Applicant has labeled the paragraphs in his response to correspond to the paragraph labeling in the Office Action for the convenience of the Examiner.

Election/Restriction Requirement

Although Applicants understand the Examiner's points, Applicants still find it difficult to understand such extensive claim restriction especially based on the patent term being set to no more than twenty years from the date of filing. Basically, Applicants are now faced with the daunting task of having to file up to 9 divisional application and incur all the related costs.

Sequence Listing

The Examiner contends as follows:

> This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821 (a)(l) and (a)(2). See for example, pages 64-67 in the specification; Figures 5, 10, and 11; and elsewhere. However, this application fails to comply with the requirements of 37 CFR § 1.821 through 1.825, because it lacks SEQ ID Nos cited along with each sequence in the specification or Figures. Applicants are also reminded that SEQ ID Nos are not required in Figures per se, however, the corresponding SEQ ID Nos then are required in the Brief Description of the Drawings section in the specification. Applicant(s) are required to submit a new computer readable form sequence listing, a paper copy, or CD-ROM for the specification, statements under 37 CFR § 1.821 (f) and (g). Applicant(s) are given the same response time regarding this failure to comply as that set forth to respond to this office action. Failure to respond to this requirement may result in abandonment of the instant application or a notice of a failure to fully respond to this Office Action.

Applicants herewith submit a revised sequence listing.

Title

The Examiner contends as follows:

> The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The present title is directed to real-time sequence determination whereas in contrast the elected claims

include *Taq* DNA polymerase I.

Applicants propose the following new title which does not specifically name Taq DNA polymerase I because Applicants believe that if a generic claim is allowable, then the Examiner must entertain Applicants' request to include other polymerases as well or other polymerizing agents as well. However, if Applicants are unable to gain allowance of a genic claim, then application will further amend the title.

Oath/Declaration

The Examiner contends as follows:

> The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.
>
> The oath or declaration is defective because the declaration contains an incorrect date for the priority document which is the provisional parent application.

Applicants are in the process of executing a new Declaration with a Power of Attorney and will forward the executed Declaration as soon as it is available.

Specification

The Examiner contends as follows:

> The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code, such as on page 5, line 3, and elsewhere. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.
>
> The disclosure is objected to because of the following informality:"att,achment" on page 37, line 12 and "Mutagensis" on page 64, line 16, are misspelled. Appropriate correction of these and any other misspellings or grammatical errors is required.

Applicants have undertaken a thorough review of the application as filed and have corrected all typographical, misspelling and grammatical errors they could find. However, Applicants do not assert that all such errors have been found.

Claim Objections

Claim 16 stands objected to for an informality.

The Examiner contends as follows:

> Claim 16 is objected to because of the following informality: "comprise" on line 1 should end in an "s." Appropriate correction is required.

Applicant traverse this rejection and request withdrawal of same based on the amendment(s) to claim 16.

Claims Rejected Under 35 U.S.C. §112, First Paragraph

Claims 6, 19, and 24 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner contends as follows:

> Claims 6,19, and 24 recite attachment site numbers ("513-518,643,647,649, and 653- 661 ") on *Taq* DNA polymerase I; however, no sequence is disclosed for *Taq* DNA polymerase I by which to determine where these sites are therein. This enzyme is noted in a reference cited on page 39, lines 5-8 of the specification, but this is essential subject matter which cannot be properly enabled by reference to a printed publication. The incorporation of essential material in the specification by reference to a foreign application or patent, or to a publication is improper. Applicant is required to amend the disclosure to include the material incorporated by reference. The amendment must be accompanied by an affidavit or declaration executed by the applicant, or a practitioner representing the applicant, stating that the amendatory material consists of the same material incorporated by reference in the referencing application. See *In re Hawkins,* 486 F.2d 569, 179 USPQ 157 (CCPA 1973); *In re Hawkins,* 486 F.2d 579, 179 USPQ 163 (CCPA 1973); and *In re Hawkins,* 486 F.2d 577,179 VSPQ 167 (CCPA 1973).

Applicants disagree and believe that **the partial amino acid sequences** listed in the patent application **are fully enabled** because they identify specific sites in the known (well-known) amino acid sequence of *Taq* DNA Polymerase I, a known and commercially available polymerase that has been fully characterized and widely used throughout the industry. Moreover, the Patel et al. reference includes only partial amino acid sequences for the *Taq* DNA Polymerase I mutants without including the complete *Taq* DNA Polymerase I sequence. Why, because ordinary artisans are fully aware of the *Taq* DNA Polymerase I amino acid sequence and fully understand mutant partial sequences that fully identify the mutant of the wild type amino acid sequence. Furthermore, Applicants have included the DNA sequences that would give rise to the *Taq* DNA Polymerase I mutants. However, Applicants have amended the specification to incorporate the non essential complete amino acid sequence of *Taq* DNA polymerase I. No new matter is added that is essential matter to support the claims because none is needed. Applicants, therefore, respectfully request withdrawal of this section 112, first paragraph rejection.

Claims Rejected Under 35 U.S.C. §112, Second Paragraph

Claims 1-24 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The Examiner contends as follows:

Claims 1-4,7-8, 10-14, 18, and 20-22 and the dependent claims therefrom contain embodiments which are beyond the elected invention, particularly "polymerizing agent" and "polymerase." Correction is suggested by stating only the embodiments (in this case, *"Taq* DNA polymerase I") which are part of the invention.

Claims 1 (line 2), 7 (line 2), and 20 (line 2) recite the phrase "located at or near" which is vague and indefinite. It is unclear what degree of closeness qualifies as being "near". Clarification of the metes and bounds of the claims via clearer claim wording is required. Claims 1, 7, and 20 are rejected along with claims 2-6, 8-9, and 21-24 due to their direct or indirect dependence therefrom.

Claims 1 (line 2), 7 (line 2), 10 (line 2), and 20 (line 2) recite the phrase "associated with" which is vague and indefinite. It is unclear what parameters are used and to what degree in order to determine that the tag is "associated with" the polymerizing agent. It is also unclear if the association is in relation to the "site" as mentioned or to the polymerizing agent as a whole. Claims 1,7, 10, and 20 are rejected along with claims 2-6,8-9, 11-19, and 21-24 due to their direct or indirect dependence therefrom.

Claims 6 (line 3), 19 (line 3), and 24 (line 3) recite the phrase "mixtures or combinations thereof of the *Taq* polymerase" which is vague and indefinite. It is unclear what else may be included in these multiple entities besides *Taq* DNA polymerase I. Clarification of the metes and bounds *of* these claims via clearer claim wording is required.

Claim 16 is vague and indefinite due to the unclarity of citing an abbreviation, such as dNTP on line 1. Correction is suggested by amending in of the full name in parentheses. Claim 18 is also rejected due to its dependency from claim 16.

Claim 16, line 2, recites the term "group" which is vague and indefinite. It is unclear which entity the "group" is a part of, the polymerase, the monomer, or the dNTP. Clarification of the location of the "group" is required. Claim 18 is also rejected due to its dependency from claim 16.

Applicants believe that the removal of the phrase "located at or near, associated with or" gives rise to a generic claim that is allowable and Applicants formally request inclusion of the complete set of polymerizing agents. Applicants have amended the claims to remove these section 112, second paragraph rejections and respectfully request withdrawal of same.

Claims Rejected - 35 U.S.C. §102

Williams/Brandis

Claims **1-5,7-18, and 20-23** are rejected under 35 U.S.C. 102(a) and (b) as being anticipated by Williams (WO 00/36151) and Brandis (1999), respectively.

The Examiner contends as follows:

Williams discloses a *Taq* DNA polymerase (p. 8, lines 23-28) in which a fluorescently labeled dNTP (tag) is associated with the polymerase during monomer incorporation (p. 8, lines 1-9). The tag consists of a labeled nucleotide triphosphate (NTP) having a γ-phosphate with a fluorophore moiety attached and a quencher moiety that sufficiently prevents fluorescence until incorporation of the NTP at which time the γ-phosphate with the fluorophore moiety is released and detected (p. 8, lines 10-20). Williams discloses the fluorescence is detected when labeled dNTPs are incorporated into the strand and fluorescence is induced (p. 9, lines 28-29). Williams discloses that upon incorporation, the fluorescent dye molecule is released with pyrophosphate from the polymerase and then swept away from the parent DNA molecule by the flow (p. 10, lines 13-17), suggesting the polymerase's detectable property reverts back to its initial state. Williams discloses that as the polymerase moves along the DNA, the nucleotide sequence is read from the order of released dyes (p. 14, lines 30-31). Williams discloses the possible presence of other polymerases, such as HIV reverse transcriptase, as stated in claims 5, 9, and 23. Thus, Williams anticipates the limitations in claims 1-5, 7-10, 17, and 20-23.

A 35 U.S.C. 102 rejection over multiple references has been held to be proper when the extra references are cited to show that a characteristic not disclosed in the reference is inherent (see MPEP 2131.01 (c)). Brandis discloses a *Taq* DNA polymerase I including an inherent characteristic that polymerases go through conformational changes (abstract). Brandis also discloses that a change occurs in a fluorescent label during the change in conformational states of the polymerase when nucleotide binding occurs as the polymerase is active (abstract). Thus, Brandis further anticipates claims 11-18 of the instant invention.

Preliminary Remarks

Claims 1-24 at issue in this application relate to three different compositions.

The first composition, claims 1-9, relates to a polymerizing agent having a tag, where a detectable property of the tag undergoes a change before, during and/or after monomer incorporation (claims 1-6) or where a detectable property of the tag has a first value when the polymerase is in a first state and a second value when the polymerase is in a second state during monomer incorporation, and where the polymerizing agent changes from the first state to the second state and back again during each monomer incorporation (claims 7-9).

The second composition, claims 10-19, relates to a polymerizing agent having a tag and a monomer having a tag, where at least one of the tags has a detectable property that undergoes a change before, during and/or after monomer incorporation due to an interaction between the polymerizing agent tag and the monomer tag.

The third composition, claims 20-24, relates to a polymerizing agent having a pair of tags, where a detectable property of at least one of the tags undergoes a change before, during and/or after monomer incorporation.

Moreover, the verbiage "located at or near, associated with or covalently bonded to" relates to tags that stay with the molecular and do not relate to tags that only transiently associate with the tagged molecule. This interpretation is made clear throughout the entire specification, where a tagged polymerase includes either two tags, such as a donor or acceptor pair, or a single tag which interacts with a tag on the dNTPs as the dNTPs are incorporated or has tags associated with associated proteins for those polymerizing agents that require activation complexes. A tagged dNTP does not satisfy the requirement of a tag "located at or near, associated with or covalently bonded to" the polymerizing agent because the tagged dNTPs only transiently associate with the polymerizing agent and is therefore not a tag that remains associated with the polymerizing agent. Applicants understand that this is a subtle distinction, but it is a critical distinction. The tag "located at or near, associated with or covalently bonded to" the polymerizing agent must remain with the polymerizing agent during all incorporation events so that a change in its property can be detected before, during or after each monomer addition; such a requirement cannot be satisfied by the tag being only on the dNTPs.

To expedite examination and allowance of this case, Applicants have amended the claims to address only the covalently bonded tags. However, Applicants believe that given the proper interpretation, the "located at or near, associated with or covalently bonded to" language is patentably distinct over the art of record.

Remarks to Anticipation

Applicants must respectfully disagree with the Examiner's assertions regarding Williams/Brandis. Williams/Brandis do not disclose a tagged polymerizing agent. The Williams method is based entirely on fluorescently tagged monomers (tagged-dNTPs) having a proximal quencher so that prior to monomer incorporation, the monomer is fluorescently inactive, while during monomer incorporation, the tag is removed from the quencher (pyrophosphate is released), dequenched, allowing fluorescence to occur.

On the contrary, all of the compositions of the present invention require a tag "located at or near, associated with or covalently bonded to" the polymerizing agent itself or require a tag "located at or near, associated with or covalently bonded to" the polymerizing agent and the monomer, where a detectable property of the at least one tag undergo a change before, during and/or after monomer incorporation. Williams/Brandis simply do not disclose tagged polymerizing agents, especially tagged *Taq* DNA polymerase I, even though *Taq* DNA polymerase I is a preferred polymerase in Williams/Brandis. Thus, Williams/Brandis do not identically disclose the present invention - no

tagging of the polymerizing agent, only tagging of the monomer. The compositions of the present invention permit increases the signal to background noise ratio, *i.e.*, improves signal to noise ratio.

Because Williams/Brandis do not tag the polymerizing agent, Williams/Brandis cannot anticipate any of the pending claims of this application. Applicants, therefore, respectfully request withdrawal of this section 102(a)/(b) rejection.

Remarks to Obviousness

Not only does Williams/Brandis not anticipate any of the pending claims of this application, they do not even render the present claims obvious. Williams/Brandis does not disclose, teach or suggest compositions including molecular and/or atomic tags "located at or near, associated with or covalently bonded to" the polymerizing agent. Both references simply use a polymerase, such as *Taq* DNA Pol I, with tagged monomers and the tagged released pyrophosphate is observed regardless of the polymerase.

Applicants therefore urge that Williams does not render claims 1-24 obvious under 35 USC section 103(a).

Claims Rejected - 35 U.S.C. §102

Patel et al.

Claims **1,3, and 4** stand rejected under 35 U.S.C. 102(e)(2) as being anticipated by Patel et al. (P/N 6,329,178).

The Examiner contends as follows:

> Patel et al. disclose the *Taq* DNA polymerase I (col. 13, lines 5-8) active site is highly mutable and can accommodate many amino acid substitutions without significantly affecting activity (col. 2, lines 63-66). Patel et al. disclose that mutant DNA polymerases can incorporate unconventional nucleotides (col. 3, lines 44-48), such as bases labeled with a reporter molecule and fluorescently labeled bases (col. 6, lines 14-19) which suggests types of tags. Patel et al. disclose the fluorescently labeled tags exhibiting different emissions when a DNA fragment is extended by DNA polymerase (col. 10, lines 38-67). Thus, Patel et al. anticipate claims 1,3, and 4.

Remarks to Anticipation

While Patel et al. do disclose and claim amino acid mutations of wild type polymerases including *Taq* DNA Pol I, Patel et al. do not disclose tagging the wild type or mutant polymerases with tags that undergo a change during monomer incorporation. The only tagging mentioned in Patel et al. is the ability for the mutant polymerases to incorporate tagged monomers.

Because Patel et al. like Williams/Brandis do not disclose tagged polymerase, wild type or

mutant, Patel et al. cannot anticipate the present invention.

Remarks to Obviousness

Patel et al. also do not render any of the pending claims obvious, because Patel et al. does not disclose, teach or suggest tagging either the wild type or mutant polymerases. Patel et al. merely discloses mutant polymerases none of which are within the regions set forth in the present application. Thus, the present mutations are not disclosed, suggested or taught by Patel et al. Moreover, Patel et al. does not disclose, teach or suggest using cysteine as the mutant amino acid, which can then be reacted with a fluorophore to tag the polymerase. Applicants therefore urge that Patel et al. do not render claims 1, 3 and 4 obvious under 35 USC section 103(a).

Having fully responded to the Examiner's Non-Final Office Action, Applicant respectfully urges that is application be passed onto allowance.

If it would be of assistance in resolving any issues in this application, the Examiner is kindly invited to contact applicant's attorney Robert W. Strozier at 713.977.7000

Date: **April 3, 2003**

Respectfully submitted,

Robert W. Strozier
Reg. No. 34,024

AMENDMENTS WITH AMENDMENT MARKINGS

Amended Page 6, lines 3-18

Conventional DNA sequencing strategies and methods are reliable, but time, labor, and cost intensive. To address these issues, some researchers are investigating fluorescence-based, single-molecule sequencing methods that use enzymatic degradation, followed by single-dNMP detection and identification. The DNA polymer containing fluorescently-labeled nucleotides is digested by an exonuclease, and the labeled nucleotides are detected and identified by flow cytometry (Davis *et al.*, 1991; Davis *et al.*, 1992; Goodwin *et al.*, 1997; Keller *et al.*, 1996; Sauer *et al.*, 1999; Werner *et al.*, 1999). This method requires that the DNA strand is synthesized to contain the fluorescently-labeled [flourescently-labeled] base(s). This requirement limits the length of sequence that can be determined, and increases the number of manipulations that must be performed before any sequence data is obtained. A related approach proposes to sequentially separate single (unlabeled) nucleotides from a strand of DNA, confine them in their original order in a solid matrix, and detect the spectroscopic emission of the separated nucleotides to reconstruct DNA sequence information (Ulmer, 1997; Mitsis and Kwagh, 1999; Dapprich, 1999). This is the approach that is being developed by Praelux, Inc., a company with a goal to develop single-molecule DNA sequencing. Theoretically, this latter method should not be as susceptible to length limitations as the former enzymatic degradation method, but it does require numerous manipulations before any sequence information can be obtained.

Amended Page 22, lines 21-27

The term monomer as used herein means any compound that can be incorporated into a growing molecular chain by a given polymerase. Such monomers include, without limitations, naturally occurring nucleotides (*e.g.*, ATP, GTP, TTP, UTP, CTP, dATP, dGTP, dTTP, dUTP, dCTP, synthetic analogs), precursors for each nucleotide, non-naturally occurring nucleotides and their precursors or any other molecule that can be incorporated into a growing polymer chain by a given polymerase. Additionally, amino acids (natural or synthetic) for protein or protein analog synthesis, mono saccharides [sacchairides] for carbohydrate synthesis or other monomeric syntheses.

Amended Page 24, lines 6-9

The present invention provides a [A] composition comprising an exonuclease including at least one molecular and/or atomic tag located at or near, associated with or covalently bonded to a site on the agent, where a detectable property has a first value when the polymerase is in a first state

and a second value when the polymerase is in a second state during monomer removal.

Amended Page 24, lines 10-13

The present invention provides a composition comprising an enzyme modified to produce a detectable response prior to, during and/or after interaction with an appropriately modified monomer, where the monomers are nucleotides, nucleotide analogs, amino acids, amino acid analogs, monosaccharides, monosaccharide analogs [monosaccarides, monosaccaride analogs] or mixtures or combinations thereof.

Amended Page 28, line 2

Cooperatively Tagged Monomers and Tagged [Polymering] Polymerizing Agent

Amended Page 28, lines 13-16

The present invention provides a composition comprising a cooperatively tagged polymerase and tagged monomers, where a detectable property of at least one of the tags changes when the tag are [within] within a distance sufficient to cause a change in the intensity and/or frequency of emitted fluorescent light.

Amended Page 31, lines 17-18

Figure 16 depicts an image of a 20% denaturing polyacrylamide [polyacryamide] gel containing size separated radiolabeled products from DNA extension experiments involving γ-ANS-phosphate-dATP;

Amended Page 31, lines 22-25

Figure 6 depicts an image of (A) 6% denaturing polyacrylamide gel, (B) a lightened phosphorimage of the actual gel, and (C) an enhanced phosphorimage of the actual gel containing products generated in DNA extension reactions using γ-ANS-phosphate-dNTPs;

Figure 7 depicts an image of (A) the actual gel, (B) a lightened phosphorimage of the actual gel, and (C) an enhanced phosphorimage of the actual gel;

Figure 8 depicts data for the Klenow fragment from *E. coli* DNA polymerase I incorporation of gamma-modified nucleotides;

Figure 9 depicts data for the *Pfu* DNA polymerase incorporation of gamma-modified nucleotides;

Figure 10 depicts data for the HIV-1 reverse transcriptase incorporation of gamma-tagged nucleotides;

Figure 11 depicts experimental results for native T7 DNA polymerase and Sequenase incorporation of gamma-tagged nucleotides; and

Figure 12 depicts reaction products produced when the four natural nucleotides (dATP, dCTP, dGTP and dTTP) are used in the synthesis reaction (solid line) and reaction products produced when base-modified nucleotides are used in the synthesis reaction.

Amended Page 32, lines 14-20

The pattern of emission signals is collected, either directly, such as by an Intensified [Intensitifed] Charge Coupled Devise (ICCD) or through an intermediate or series of intermediates to amplify signal prior to electronic detection, where the signals are decoded and confidence values are assigned to each base to reveal the sequence complementary to that of the template. Thus, the present invention also provides techniques for amplifying the fluorescent light emitted from a fluorescent tag using physical light amplification techniques or molecular cascading agent to amplify the light produced by single-molecular fluorescent events.

Amended Page 35, lines 17-24

In one embodiment of the single-molecule DNA sequencing system of this invention, a single tag is attached to an appropriate site on a polymerase and a unique tag is attached to each of the four nucleotides: dATP, dTTP, dCTP and dGTP. The tags on each dNTPs are designed to have a unique emission signature (*i.e.*[*i,e,*], different emission frequency spectrum or color), which is directly detected upon incorporation. As a tagged dNTP is incorporated into a growing DNA polymer, a characteristic fluorescent signal or base emission signature is emitted due to the interaction of polymerase tag and the dNTP tag. The fluorescent signals, *i.e.*, the emission intensity and/or frequency, are then detected and analyzed to determine DNA base sequence.

Amended Page 37 line 11 to Page 38 line 29

Suitable atomic tag for use in this invention include, without limitation, any atomic element amenable to attachment [att,achment] to a specific site in a polymerizing agent or dNTP, especially fluorescent dyes such as d-Rhodamine acceptor dyes including dichloro[R110], dichloro[R6G], dichloro[TAMRA], dichloro[ROX] or the like, fluorescein donor dye including fluorescein, 6-FAM, or the like;_Acridine including Acridine orange, Acridine yellow, Proflavin, pH 7, or the like; Aromatic Hydrocarbon including 2-Methylbenzoxazole, Ethyl p-dimethylaminobenzoate, Phenol, Pyrrole, benzene, toluene, or the like; Arylmethine Dyes including Auramine O, Crystal violet, H2O, Crystal violet, glycerol, Malachite Green or the like; Coumarin dyes including 7-Methoxycoumarin-4-acetic acid, Coumarin 1, Coumarin 30, Coumarin 314, Coumarin 343, Coumarin 6 or the like; Cyanine Dye including 1,1'-diethyl-2,2'-cyanine iodide, Cryptocyanine, Indocarbocyanine (C3)dye, Indodicarbocyanine (C5)dye, Indotricarbocyanine (C7)dye,

Oxacarbocyanine (C3)dye, Oxadicarbocyanine (C5)dye, Oxatricarbocyanine (C7)dye, Pinacyanol iodide, Stains all, Thiacarbocyanine (C3)dye, ethanol, Thiacarbocyanine (C3)dye, n-propanol, Thiadicarbocyanine (C5)dye, Thiatricarbocyanine (C7)dye, or the like; Dipyrrin dyes including N,N'-Difluoroboryl-1,9-dimethyl-5-(4-iodophenyl)-dipyrrin, N,N'-Difluoroboryl-1,9-dimethyl-5-[(4-(2-trimethylsilylethynyl), N,N'-Difluoroboryl-1,9-dimethyl-5-phenydipyrrin, or the like; Merocyanines including 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), acetonitrile, 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), methanol, 4-Dimethylamino-4'-nitrostilbene, M erocyanine 540, or the like; Miscellaneous Dye including 4',6-Diamidino-2-phenylindole (DAPI), 4',6-Diamidino-2-phenylindole (DAPI), dimethylsulfoxide, 7-Benzylamino-4-nitrobenz-2-oxa-1,3-diazole, Dansyl glycine, H2O, Dansyl glycine, dioxane, Hoechst 33258, DMF, Hoechst 33258, H2O, Lucifer yellow CH, Piroxicam, Quinine sulfate, 0.05 M H2SO4, Quinine sulfate, 0.5 M H2SO4, Squarylium dye III, or the like; Oligophenylenes i ncluding 2 ,5-Diphenyloxazole (PPO), B iphenyl, P OPOP, p -Quaterphenyl, p -Terphenyl, or the like; Oxazines including Cresyl violet perchlorate, Nile Blue, methanol, Nile Red, Nile blue, ethanol, Oxazine 1, Oxazine 170, or the like; Polycyclic Aromatic Hydrocarbons including 9,10-Bis(phenylethynyl)anthracene, 9,10-Diphenylanthracene, Anthracene, Naphthalene, Perylene, Pyrene, or the like; polyene/polyynes including 1,2-diphenylacetylene, 1,4-diphenylbutadiene, 1,4-diphenylbutadiyne, 1,6-Diphenylhexatriene, Beta-carotene, Stilbene, or the like; Redox-active Chromophores including Anthraquinone, Azobenzene, Benzoquinone, Ferrocene, Riboflavin, Tris(2,2'-bipyridyl)ruthenium(II), Tetrapyrrole, Bilirubin, Chlorophyll a, diethyl ether, Chlorophyll a, methanol, Chlorophyll b, Diprotonated-tetraphenylporphyrin, Hematin, Magnesium octaethylporphyrin, Magnesium octaethylporphyrin (MgOEP), Magnesium phthalocyanine (MgPc), PrOH, Magnesium phthalocyanine (MgPc), pyridine, Magnesium tetramesitylporphyrin (MgTMP), Magnesium tetraphenylporphyrin (MgTPP), O ctaethylporphyrin, P hthalocyanine (Pc), P orphin, Tetra-t-butylazaporphine, Tetra-t-butylnaphthalocyanine, Tetrakis(2,6-dichlorophenyl)porphyrin, Tetrakis(o-aminophenyl)porphyrin, Tetramesitylporphyrin (TMP), Tetraphenylporphyrin (TPP), Vitamin B12, Zinc octaethylporphyrin (ZnOEP), Zinc phthalocyanine (ZnPc), pyridine, Zinc tetramesitylporphyrin (ZnTMP), Zinc tetramesitylporphyrin radical cation, Zinc tetraphenylporphyrin (ZnTPP), or the like; Xanthenes including Eosin Y, Fluorescein, basic ethanol, Fluorescein, ethanol, Rhodamine 123, Rhodamine 6G, Rhodamine B, Rose bengal, Sulforhodamine 101, or the like; or mixtures or combination thereof o r sy nthetic derivatives thereof or FRET fluorophore-quencher pairs including DLO-FB1 (5'-FAM/3'-BHQ-1) DLO-TEB1 (5'-TET/3'-BHQ-

1), DLO-JB1 (5'-JOE/3'-BHQ-1), DLO-HB1 (5'-HEX/3'-BHQ-1), DLO-C3B2 (5'-Cy3/3'-BHQ-2), DLO-TAB2 (5'-TAMRA/3'-BHQ-2), DLO-RB2 (5'-ROX/3'-BHQ-2), DLO-C5B3 (5'-Cy5/3'-BHQ-3), DLO-C55B3 (5'-Cy5.5/3'-BHQ-3), MBO-FB1 (5'-FAM/3'-BHQ-1), MBO-TEB1 (5'-TET/3'-BHQ-1), MBO-JB1 (5'-JOE/3'-BHQ-1), MBO-HB1 (5'-HEX/3'-BHQ-1), MBO-C3B2 (5'-Cy3/3'-BHQ-2), MBO-TAB2 (5'-TAMRA/3'-BHQ-2), MBO-RB2 (5'-ROX/3'-BHQ-2); MBO-C5B3 (5'-Cy5/3'-BHQ-3), MBO-C55B3 (5'-Cy5.5/3'-BHQ-3) or similar FRET pairs available from Biosearch Technologies, Inc. of Novato, CA, tags with nmr active groups, tags with spectral features that can be easily identified such as IR, far IR, visible UV, far UV or the like.

Amended Page 46, lines 13

Amino [Acide] Acid Site Selection for the *Taq* Polymerase

Amended Page 54, lines 23-31

The present invention uses tagged dNTPs or ddNTPs in combination with polymerase for signal detection. The dNTPs are modified at phosphate positions (alpha, beta and/or gamma) and/or other positions of nucleotides through a covalent bond or affinity association. The tags are designed to be removed from the base before the next monomer is added to the sequence. One method for removing the tag is to place the tag on the gamma and/or beta phosphates. The tag is removed as pyrophosphate [pyrrophosphate] dissociates from the growing DNA sequence. Another method is to attach the tag to a position of on the monomer through a cleavable bond. The tag is then removed after incorporation and before the next monomer incorporation cleaving the cleavable bond using light, a chemical bond cleaving reagent in the polymerization medium, and/or heat.

Amended Page 55, lines 6-12

where FR is a fluorescent tag, L is a linker group, X is either H or a counterion [counterion] depending on the pH of the reaction medium, Z is a group capable of reaction with the hydroxyl group of the pyrophosphate and Z' is group after reaction with the dNMP. Preferably, Z is Cl, Br, I, OH, SH, NH_2, NHR, CO_2H, CO_2R, SiOH, SiOR, GeOH, GeOR, or similar reactive functional groups, where R is an alkyl, aryl, aralkyl, alkaryl, halogenated analogs thereof or hetero atom analogs thereof and Z' is O, NH, NR, CO_2, SiO, GeO, where R is an alkyl, aryl, aralkyl, alkaryl, halogenated analogs thereof or hetero atom analogs thereof[ ].

Amended Page 57 line27 to Page 58 line 2

TABLE V

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Primer Strand:
     TOP   5' GGT ACT AAG CGG CCG CAT G 3'          (SEQ ID NO. 1)
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Template Strands:

BOT-T 3' CCA TGA TTC GCC GGC GTA CTC 5' (SEQ ID NO. 2)
BOT-C -3' CCA TGA TTC GCC GGC GTA CCC 5' (SEQ ID NO. 3)
BOT-G 3' CCA TGA TTC GCC GGC GTA CGC 5' (SEQ ID NO. 4)
BOT-A 3' CCA TGA TTC GCC GGC GTA CAC 5' (SEQ ID NO. 5)
BOT-3T 3' CCA TGA TTC GCC GGC GTA CTT TC 5' (SEQ ID NO. 6)
BOT-Sau 3' CCA TGA TTC GCC GGC GTA CCT AG 5' (SEQ ID NO. 7)

Amended Page 64, lines 1-10

Taq Pol I forward

5'-gc gaattc atgaggggga tgctgcccct ctttgagccc-3' (SEQ ID NO. 8)

Taq Pol I reverse

5'-gc gaattc accctccttgg cggagcgc cagtcctccc-3' (SEQ ID NO. 9)

The underlined segment of each synthetic DNA oligonucleotide represents engineered EcoRI restriction sites immediately preceding and following the *Taq* pol I gene. PCR amplification using the reverse primer described above and the following forward primer created an additional construct with an N-terminal deletion of the gene:

Taq Pol I_A293_trunk

5'-aatccatgggccctggaggaggc cccctggccccgc-3' (SEQ ID NO. 10)

Amended Page 64, line 16-21

Mutagenesis [Mutagensis]

Once a suitable construct is generated, individual cysteine mutations are introduced at preferred amino acid positions including positions 513-518, 643, 647, 649 and 653-661 of the native *Taq* polymerase having the following amino acid sequence:

1 mrgmlplfep kgrvllvdgh hlayrtfhal kqlttsrgep vqavygfaks llkalkedgd
61 avivvfdaka psfrheaygg ykaqraptpe dfprqlalik elvdllglar levpgyeadd
121 vlaslakkae keqyevrilt adkdlyqlls drihvlhpeg ylitpawlwe kyglrpdqwa
181 dyraltgdes dnlpgvkgig ektarkllee wgsleallkn ldrlkpaire kilahmddlk
241 lswdlakvrt dlplevdfak rrepdrerlr aflerlefgs llhefglles pkaleeapwp
301 ppegafvgfv lsrkepmwad llalaaargg rvhrapepyk alrdlkearg llakdlsvla
361 lreglglppg ddpmllayll dpsnttpegv arryggewte eageraalse rlfanlwgrl
421 egeerllwly reverplsav lahmeatgvr ldvaylrals levaeeiarl eaevfrlagh
481 pfnlnsrdql ervlfdelgl paigktektg krstsaavle alreahpive kilqyreltk
541 lkstyidplp dlihprtgrl htrfnqtata tgrlsssdpn lqnipvrtpl ggrirrafia
601 eegwllvald ysqielrvla hlsqdenlir vfqegrdiht etaswmfgvp reavdplmrr
661 aaktinfgvl ygmsahrlsg elaipyeeag afieryfgsf pkvrawiekt leegrrrgyv

721 etlfqrrryv pdlearvksv reaaermafn mpvqgtaadl mklamvklfp rleemgarml

781 lqvhdelvle apkeraeava rlakevmeqv yplavpleve vqiqedwlsa ke (SEQ ID NO. 11).

The following amino acid residues correspond to the amino acids between amino acid 643 and 661, where xxx represents intervening amino acid residues in the native polymerase:

643-Ala xxx xxx xxx Phe xxx Val xxx xxx Glu Ala Val Asp Pro Leu Met Arg Arg Ala -661 (SEQ ID NO. 12).

Amended Pages 65 line 1 to Page 67 line 1

Alanine 643 to Cysteine Replacement

Taq Pol I_Ala643Cys_fwd

5'-C CAC ACG GAG ACC tgC AGC TGG ATG TTC GGC G-3' (SEQ ID NO. 13)

Taq Pol I_Ala643Cys_rev

5'-C GCC GAA CAT CCA CGA Gca GGT CTC CGT GTG G-3' (SEQ ID NO. 14)

Phenylalanine 647 to Cysteine Replacement

Taq Pol I_Phe647Cys_fwd

5'-CC GCC AGC TGG ATG TgC GGC GTC CCC CGG GAG GCC-3' (SEQ ID NO. 15)

Taq Pol I_Phe647Cys_rev

5'-GGC CTC CCG GGG GAC GCC GcA CAT CCA CGT GGC GG-3' (SEQ ID NO. 16)

Valine 649 to Cysteine Replacement

Taq Pol I_Val649Cys_fwd

5'-GCC AGC TGG ATG TTC GGC tgC CCC CGG GAG GCC GTG G-3' (SEQ ID NO. 17)

Taq Pol I_Val649Cys_rev

5'-C CAC GGC CTC CCG GGG Gca GCC GAA CAT CCA GCT GGC-3' (SEQ ID NO. 18)

Glutamic Acid 652 to Cysteine Replacement

Taq Pol I_Glu652Cys_fwd

5'-GGC GTC CCC CGG tgc GCC GTG GAC CCC CTG ATG CGC-3' (SEQ ID NO. 19)

Taq PolI_Glu652Cys_rev

5'-GCG CAT CAG GGG GTC CAC GGC gca CCG GGG GAC GCC-3' (SEQ ID NO. 20)

Alanine 653 to Cysteine Replacement

Taq Pol I_Ala653Cys_fwd

5'-GGC GTC CCC CGG GAG tgC GTG GAC CCC CTG ATG CGC-3' (SEQ ID NO. 21)

Taq Pol I_Ala653Cys_rev

5'-GCG CAT CAG GGG GTC CAC Gca CTC CCG GGG GAC GCC-3' (SEQ ID NO. 22)

Valine 654 to Cysteine Replacement

Taq Pol I_Val654Cys_fwd

5'-GTC CCC CGG GAG GCC tgt GAC CCC CTG ATG CGC-3' (SEQ ID NO. 23)

Taq PolI_Val654Cys_rev

5'-GCG CAT CAG GGG GTC aca GGC CTC CCG GGG GAC-3' (SEQ ID NO. 24)

Aspartic Acid 655 to Cysteine Replacement

Taq Pol I_D655C_fwd

5'-CCC CGG GAG GCC GTG tgC CCC CTG ATG CGC CGG-3' (SEQ ID NO. 25)

Taq Pol I_D655C_rev

5'-CCG GCG CAT CAG GGG Gca CAC GGC CTC CCG GGG-3' (SEQ ID NO. 26)

Proline 656 to Cysteine Replacement

Taq Pol I_Pro656Cys_fwd

5'-CGG GAG GCC GTG GAC tgC CTG ATG CGC CGG GCG-3' (SEQ ID NO. 27)

Taq Pol I_Pro656Cys_rev

5'-CGC CCG GCG CAT CAG Gca GTC CAC GGC CTC CCG-3' (SEQ ID NO. 28)

Leucine 657 to Cysteine Replacement

Taq Pol I_Leu657Cys_fwd

5'-GCC GTG GAC CCC tgc ATG CGC CGG GCG GCC-3' (SEQ ID NO. 29)

Taq Pol I_Leu657Cys_rev

5'-GGC CGC CCG GCG CAT gca GGG GTC CAC GGC-3' (SEQ ID NO. 30)

Methionine 658 to Cysteine Replacement

Taq Pol I_Met658Cys_fwd

5'-GCC GTG GAC CCC CTG tgt CGC CGG GCG GCC-3' (SEQ ID NO. 31)

Taq Pol I_Met658Cys_rev

5'-GGC CGC CCG GCG aca CAG GGG GTC CAC GGC-3' (SEQ ID NO. 32)

Arginine 659 to Cysteine Replacement

Taq Pol I_Arg659Cys_fwd

5'-GCC GTG GAC CCC CTG ATG tGC CGG GCG GCC AAG ACC-3' (SEQ ID NO. 33)

Taq Pol I_Arg659Cys_rev

5'-GGT CTT GGC CGC CCG GCa CAT CAG GGG GTC CAC GGC-3' (SEQ ID NO. 34)

Arginine 660 to Cysteine Replacement

Taq Pol I_Arg660Cys_fwd

5'-GAC CCC CTG ATG CGC tGc GCG GCC AAG ACC ATC-3' (SEQ ID NO. 35)

Taq Pol I_Arg660Cys_rev

5'-GAT GGT CTT GGC CGC gCa GCG CAT CAG GGG GTC-3' (SEQ ID NO. 36)

Alanine 661 to Cysteine Replacement

Taq Pol I_Ala661Cys_fwd

5'-CCC CTG ATG CGC CGG tgc GCC AAG ACC ATC AAC-3' (SEQ ID NO. 37)

Taq Pol I_Ala661Cys_rev

5'-GTT GAT GGT CTT GGC gca CCG GCG CAT CAG GGG-3' (SEQ ID NO. 38)

Amended Page 68 line 20 to Page 69 line 7

In the first example, illustrates the incorporation of ANS-γ-phosphate dATP to produce extended DNA products from primer templates. The reactions were carried out in extension buffer and the resulting Radiolabeled products were size separated on a 20% denaturing polyacrylamide [polyacryamide] gel. Data was collected using a phosphorimaging system. Referring now the Figure 13, **Lane 1** contains 5' radiolabeled 'TOP' probe in extension buffer. **Lane 2** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 3** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 4** contains *Taq* DNA polymerase, 50 μM ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 5** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 6** contains spill-over from lane 5. **Lane 7** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 8** contains *Taq* DNA polymerase, 50 μM ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 9** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 10** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 11** contains *Taq* DNA polymerase, ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 12** contains 5' radiolabeled 'TOP' probe in extension buffer. **Lane 13** contains 5' radiolabeled 'TOP' probe and *Taq* DNA polymerase in extension buffer. Oligonucleotide sequences are shown in Table V.

Amended Page 69 line21 to Page 70 line 20

This next example illustrates the synthesis of extended DNA polymers using all four ANS

tagged γ-phosphate dNTPs. Products generated in these reactions were separated on a 20% denaturing polyacrylamide gel, the gel was dried and imaged following overnight exposure to a Fuji BAS1000 imaging plate. Referring now to Figure 14, an image of (A) the actual gel, (B) a lightened phosphorimage and (C)) an enhanced phosphorimage. Lane descriptions for A, B, and C follow: **Lane 1** is the control containing purified 10-base primer extended to 11 and 12 bases by template-mediated addition of alpha-^{32}P dCTP. **Lane 2** includes the same primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes (to denature template), the reaction was brought to 37°C (to anneal primer-template), *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 3** includes the same labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was DNA polymerase and all four gamma-modified dNTPs (100 μ[u]M, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 4** includes the control, purified 10-base primer that was extended to 11 and 12 bases by the addition of alpha-^{32}P-dCTP was cycled in parallel with lanes 5-8 reactions. **Lane 5** includes the same ^{32}P-labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 6** includes the same ^{32}P-labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 7** includes nonpurified, 10-base, ^{32}P-labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 8** includes nonpurified, 10-base, ^{32}P-labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. Evident in the reactions involving tagged dNTPs is a substantial decrease in pyrophosphorolysis as compared to reactions involving natural nucleotides.

Clean Copy of Amended Page 70 line 21 to Page 71 line 21

This next example illustrates the synthesis of long DNA polymers using all four ANS tagged γ-phosphate dNTPs. Each primer extension reaction was split into two fractions, and one fraction was electrophoresed through a 20% denaturing gel (as described above), while the other was electrophoresed through a 6% denaturing gel to better estimate product lengths. The gel was dried and imaged (overnight) to a Fuji BAS1000 imaging plate. Referring now to Figure 15, an image of (A) the actual gel, (B) a lightened phosphorimage of the actual gel, and (C)) an enhanced phosphorimage of the actual gel. Lane descriptions for A, B, and C follow: **Lane 1** includes 123 Marker with size standards indicated at the left of each panel. **Lane 2** contains the control, purified 10-base primer extended to 11 and 12 bases by template-mediated addition of alpha-^{32}P dCTP. **Lane 3** contains the same ^{32}P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes (to denature template), the reaction was brought to 37°C (to anneal primer-template), *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 4** includes the same ^{32}P -labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C, *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μ[u]M, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 5** includes the control, purified 10-base primer that was extended to 11 and 12 bases by the addition of alpha-^{32}P -dCTP was cycled in parallel with lanes 5-8 reactions. **Lane 6** includes the same ^{32}P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 7** includes the same ^{32}P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 8** includes nonpurified, 10-base, ^{32}P -labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μ[u]M, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 9** includes nonpurified, 10-base, ^{32}P -labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs were added.

The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes.

Amended Page 72, lines 14-19

Thus, for the Taq polymerase or the HIV1 reverse transcriptase, improved fidelity, due to the use of the gamma-modified dNTPs of this invention, enables single-molecule DNA sequencing. However, not all polymerases equally utilize the gamma-modified[modifed] nucleotides of this invention, specifically, Klenow, Sequenase, HIV-1 reverse transcriptase and Taq polymerases incorporate the modified [modifed] nucleotides of this invention, while the *Pfu* DNA polymerase does not appear to incorporate the modified nucleotides of this invention.

In the Claims

1.(**amended**) A composition comprising a polymerizing agent including at least one molecular and/or atomic tag [located at or near, associated with or] covalently bonded to a site on the polymerizing agent, where a detectable property of the tag undergoes a change before, during and/or after monomer incorporation.

7.(**amended**) A composition comprising a polymerase or reverse transcriptase including at least one molecular and/or atomic tag [located at or near, associated with or] covalently bonded to a site on the polymerase, where a detectable property has a first value when the polymerase is in a first state and a second value when the polymerase is in a second state during monomer incorporation, and where the polymerizing agent changes from the first state to the second state and back again during each monomer incorporation.

10.(**amended**) A composition comprising a polymerizing agent including a molecular and/or atomic tag [associated with or] covalently bonded to a site on the polymerase and a monomer including a molecular and/or atomic tag, where at least one of the tags has a detectable property that undergoes a change before, during and/or after monomer incorporation due to an interaction between the polymerizing agent tag and the monomer tag.

16.(**amended**) The composition of claim 12, wherein the monomer comprises a dNTP and the tag is covalently bonded to the β or γ phosphate group.

20.(**amended**) A composition comprising a polymerase or reverse transcriptase including a pair of tags [located at or near, associated with or] covalently bonded to a site [of] on the polymerase, where a detectable property of at least one of the tags undergoes a change before, during and/or after monomer incorporation.